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An association between abnormal vaginal flora during pregnancy and the risk for early-onset neonatal infection

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
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Summary

Background: The main aim of his study was to determine the relationship between lower genital tract genital mycoplasmas and BV as diagnosed during pregnancy early-onset neonatal infection.

Material/Methods: The study population comprised 120 pregnant women between 22 and 36 weeks of pregnancy. The vaginal swabs were testing for diagnosis of Bacterial vaginosis (BV) by Gram's stain method according to Spiegel's criteria. Cervical swabs were collected for isolation and identification of genital mycoplasmas. Early-onset neonatal infection was diagnosed on clinical and laboratory symptoms of the newborns.

Results: Among 120 examined pregnant women BV was diagnosed in 32 (26.7%)women, 35 (29.1%) had intermediate flora and among 53 (44.2%) normal flora was diagnosed. Genital mycoplasmas was diagnosed in 37 subject (30.8%). 21 (17.5%) of the 120 study women delivered newborns with early-onset neonatal infection (EONI) (group I), while 99 (82.5%) constituted reference group (non-IW – group II). Co-infection of BV with mycoplasmas or genital mycoplasmas infection diagnosed at midgestation constituted an important risk for delivering an infant with early-onset neonatal infection (OR =7.11, and OR =4.44, respectively).

Conclusions: Bacterial vaginosis and the lower genital tract colonization by Mycoplasma hominis and Ureaplasma urealyticum between 22 and 36 weeks of gestation constitute risk factors for early-onset neonatal infection what indicate the need for detection and monitoring of this infections among pregnant women even before pregnancy.

Key words: **bacterial vaginosis • genital mycoplasmas • risk factors • early-onset neonatal infection**

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Background

Early-onset neonatal infection are one of the most common perinatal complication. Four possible mechanisms exist for microbes to spread to the uterus, an otherwise sterile environment: (i) the organisms originate from elsewhere and are transmitted hematogenously; (ii) the organisms from the peritoneal cavity seed retrogradely through the fallopian tube; (iii) they are inoculated accidentially inside the uterus during invasive procedures, such as amniocentesis and chorionic villous sampling, and (iv) the organisms from vagina and the cervix ascend to the uterus. Ascending infection is considered to be by far the most common route of infection [1].

Abnormal microbiological flora of the lower genital tract – Bacterial vaginosis (BV) is clinical syndrome definied as a decrease in the normally occurring lactobacillus species in the vagina and an increase in other predominantly anaerobic organismas, including *Gardnerella vaginalis, Bacteroides spp, Prevotella spp, Mobiluncus spp, Fusobacterium spp,* and genital mycoplasmas – *Mycoplasma hominis* and/or *Ureaplasma urealyticum* [2,3].

Bacterial vaginosis associated flora are well knows cause of perinatal complication. It has been documented that BV and/or genital mycoplasmas are an important risk factors for chorioamnionitis and amniotic fluid (AF) infection [4–10]. Although BV associated flora and genital mycoplasmas have been isolated as a infectious agent from amniotic fluid among pregnant women, a casual relationship with colonization of lower genital tract in early pregnancy and early-onset neonatal infection in newborns are still controversial.

The aim of this study was to determine whether lower genital tract genital mycoplasmas infections and BV, as diagnosed between 22 and 36 weeks of pregnancy, are associated with early-onset neonatal infection

Material and Methods

Study population

The study population comprised 120 women at 22 to 34 weeks' gestation recruited from the patients of the clinical hospital at the Department of Perinatoloy, Medical University of Łódź, Poland, between May 2001 and December 2002. Only the singleton pregnancies were qualified for inclusion in the survey. The exclusion criteria were as follows: antibiotic therapy within 4 weeks prior to the examination, multiple gestation, vaginal bleeding, serious maternal diseases and any immunological disorders. Of 120 women enrolled six women were lost to follow up and excluded from further analysis so final study group comprised 114 women.

This prospective cohort study was approved by the Biomedical Ethics Committee of the Medical University of Łódź, Poland. (Decision No. RNN/215/00). Each participant provided a written informed consent to participate in the study.

A standard questionnaire covering medical, socio-economic, demographic and constitutional aspects as well as tobacco smoking was administered to every subject and verified based on medical records. Routine ultrasound examination of fetal biometry was performed. The gestational age at the time of sampling was based on the time of last menstruation and verified by early ultrasound (CRL – crown-rump length) of the fetus.

Bacteriological examination

For the assessment of biocenosis, vaginal and cervical swabs were collected from all pregnant women under study. The vaginal swabs were testing for Bacterial vaginosis (BV). Bacterial vaginosis was diagnosed by Gram's stain according to Spiegel's criteria [11], and the flora were graded as follows:

Grade I – normal – predominantly lactobacillus morphotypes;
Grade II – intermediate – mixed lactobacillus and other Gram-positive and Gram-negative bacterial morphotypes;
Grade III – bacterial vaginosis (BV) – few or absent lactobacillus morphotypes, but greatly increased number of *G.vaginalis* and other anaerobic Gram-negative roads.

Cervical swabs were collected for isolation and identification of genital mycoplasmas (commercially available Mycoplasma DUO kits, Sanofi Diagnostics Pasteur were used). Identification was based on specific hydrolysis of urea (*U.urealyticum*) or arginine (*M.hominis*) by the species present in specimen, which is indicated by change in the colour of the well containing the substrate, without clouding in the medium.

Early-onset neonatal infection

The early-onset neonatal infection (EONI) defined as infections occurring within first 48 hours of birth. The EONI was defined as presence of 3 of the following clinical signs suggestive of infection: lethargy or poor feeding; axillary temperature <36ºC or >38º C for more than one hour; significant jaundice with serum bilirubin >15 mg% in the absence of blood group incompatibility; apnoea or respiratory distress; peripheral capillary refill time of >3 sec on the forehead or mid sternum; heart rate of >160/min corrected for elevation of body temperature (10 beats/1ºC rise); vomiting, diarrhoea or ileus; petechiae or bleeding diathesis; omphalitis; seizures and 1 of the following laboratory markers: total leukocyte count <5,000/mm^3, neutrophil count <1,500/mm^3, and immature to total neutrophil ratio >0.2.

Statictical analysis

For comparison of prevalence of genital mycoplasmas and BV_2 χ^2 was used. Odds ratios (OR) were calculated to evaluate risk factors. Statistically analysis was conducted using EPI INFO software, taking into account the odds ratios and 95% confidence intervals (CI). Statistical analysis was carried out using STATA 8 software

Results

The mean gestational age at the time of microbiological smplings was 29.0 (SD 4.0) weeks. The mean maternal age of the study group was 27.3 (SD 4.7) years. 21% of the subject

Table 1. The prevalence of BV and genital mycoplasmas among women who delivered infants with early-onset neonatal infection (EONI) and among women who delivered infants without early-onset neonatal infection (non EONI).

Gram-stain mycoplasmas	EONI (n=21)		non EONI (n=99)		p
BV	9	42.9%	23	23.2%	ns
Intermediate flora	5	23.8%	30	30.3%	ns
Normal flora	7	33.3%	46	46.5%	**ns**
mycoplasmas	13	61.9%	24	24.2%	**p<0.01**

Table 2. The relation in prevalence of BV and genital mycoplasmas.

Gram-stain mycoplasmas	EONI (n=21)		non EONI (n=99)		p
BV	2	9.5%	11	11.1%	ns
BV and mycoplasmas	8	38.0%	12	12.1%	**p<0.01**
Mycoplasmas and intermediate flora	2	9.5%	5	5.5%	ns
Mycoplasmas and normal flora	3	14.3%	7	7.1%	ns
Intermediate and normal flora	6	28.6%	64	64.6%	**p<0.01**

Table 3. Association between BV and genital mycoplasmas diagnosed at 22 weeks gestation and the risk of delivering infant with early-onset neonatal infection.

	EONI				OR 95%CI
	+ (n=21)		– (n=99)		
BV(–) mycoplasmas (–)	6	28.6%	64	64.6%	Reference group
BV(–) mycoplasmas (+)	5	23.8%	12	10.0%	4.44 (0.90–20.42)
BV(+) mycoplasmas (–)	2	9.52%	11	11.1%	1.94 (0.17–12.72)
BV(+) mycoplasmas (+)	8	38.1%	12	12.1%	7.11 (1.81–29.05)

were unmarried and 16.1% had primary education. In the study population, 60.5% of women were nulliparous and 15.8% were smoking during pregnancy.

Microbiological and clinical characteristic

Among 120 examined pregnant women BV was diagnosed in 32 (26.7%) women, 35 (29.1%) had intermediate flora and among 53 (44.2%) normal flora was diagnosed. Genital mycoplasmas was diagnosed in 37 subject (30.8%).

21 (17.5%) of the 120 study women delivered newborns with early-onset neonatal infection (EONI) (group I), while 99 (82.5%) constituted reference group (non-EONI – group II).

Microbiological results and early-onset neonatal infection

Women who delivered infants with EONI were more likely to be BV and genital mycoplasmas positive (both *M.hominis* and *U.urealyticum* or alone) than those in references group. 9 (42.9%) of women of group I had BV and 13 (61.9%) were culture-positive for genital mycoplasmas as compared to 23 (23.2%) and 24 (24.2%, p=0.033) in the group II (Table 1).

We also evaluated the relation in prevalence of BV and genital mycoplasmas between women from group I and group II. BV was diagnosed alone in 2 (9.5%) cases from group I and in 11 (11.1%) cases from group II. The most frequently co-infection BV/mycoplasmas were diagnosed in group I – 8 (38.1) as compared to 12 women (12.1%) from the group II (Table 2).

Co-infection BV/mycoplasmas or genital mycoplasmas infection diagnosed during pregnancy constituted an important risk for delivering an infant with early-onset neonatal infection (OR =7.11, and OR =4.44; respectively) (Table 3).

Discussion

The prevalence of bacterial vaginosis among pregnant women ranges from 12% to 50%, depending on the population

studied [12–14]. In our study BV was diagnosed among 26.7% of pregnant women at 22–36 weeks of gestation by Gram's stain using Spiegel's criteria. The relatively high incidence of BV could be explained by use of sensitive diagnostic method. In clinical practice bacterial vaginosis is usually diagnosedon the basis of composite Amsel's criteria. According to Tam et al. [15] sensitivity of Gram stain method was significantly higher than that of clinical criteria (91% vs 46%). The Gram stain method has both a low false-negative (4%) and high negative predictive values (96%).

It was shown, that *Mycoplasma hominis* and *Ureaplasma urealyticum* are the most common organisms isolated in the perinatal period and are associated with the more frequent occurrence of bacterial vaginosis [16–19]. In our study genital mycoplasmas were isolated among 30% of the 120 pregnant women and among 62.5% of women with BV.

Bacterial vaginosis associated flora and genital mycoplasmas are well knows causes of intraamniotic infection. A variety of microorganisms have been isolated from the placenta, amniotic fluid and chorioamnion cultures [20–22]. The most common microorganisms involved in intrauterine infections are *Ureaplasma urealyticum, Mycoplasma hominis* and other bacterial vaginosis associated flora, especially *Fusobacterium* and *Bacteroides species.* Hitti et al. [23] isolated the same microorganisms from amniotic fluid (AF) culture and vagina. 72% of the women with positive AF culture had bacterial vaginosis-associated flora isolated from AF. Amniotic fluid infection was associated with absence of hydrogen peroxide-producing *Lactobacillus,* and presence of vaginal *Bacteroides urealyticus* and *Fusobacterium spp.* Jacobson et al. [24] investigated the occurrence of amniotic fluid infection caused by genital *M.hominis* and *U.urealyticum* and anaerobic bacteria in population of Swedish women with preterm prelabor rupture of membranes. Microorganisms in amniotic fluid were detected in 25% patients. Rzanek-Głowacka et al. [25] evaluated the relationship between bacterial flora taken from cervix of pregnant women with premature rupture of membranes (PROM) and presence of clinical and labolatory symptoms of infections of the newborn. On the basis on analyzed values PROM among mothers with bacterial vaginosis was the important risk factor of the early-onset neonatal infection.

In our study bacterial vaginosis and genital mycoplasmas have been associated with vaginal colonization in women at 22–36 weeks' gestation and constituted a significant risk factors for delivering infant with early-onset neonatal infection (OR = 7.11 CI: 1.81–29.05). BV was detected in almost half of the women (42.9%) and genital mycoplasmas among 57.4% women who delivered infants with early-onset neonatal infection. Povlsen et al. [26] have reported that *U. urealyticum* may be the cause infection independently of bacterial vaginosis. We also detected BV in 9.52% of women without mycoplasmas infection and *M.hominis and/or* U. urealyticum without sings of BV.

Many studies have shown that early-onset neonatal infection are highly prevalent among women who have preterm labor. The presence of microorganisms in amniotic fluid usually results in spontaneous abortion, prematurity and fetal or neonatal infections. AF infection occurs in 10–15% of pregnancies complicated by preterm labor [27–31]. It has been demonstrated that some microbes are able to cross the intact membranes, however, asymptomatic women with intact membranes may also sometimes (incidence to 24%) have bacteria in their amniotic fluid [32,33]. In previous study we also documented that bacterial vaginosis and genital mycoplasmas in early pregnancy are the risk factors for low birth weight and preterm delivery [34–36].

The results of this study suggest that bacterial vaginosis and lower genital tract colonization by *Mycoplasma hominis* and *Ureaplasma urealyticum,* as diagnosed at midgestation constitute risk factors for early-onset neonatal infection what indicate the need for detectable and monitoring of pregnant women even before pregnancy for thease abnormal bacterial flora. This could indicate the need for detection and monitoring of this infections among pregnant women early at or even before pregnancy.

Conclusions

Bacterial vaginosis and the lower genital tract colonization by Mycoplasma hominis and Ureaplasma urealyticum between 22 and 36 weeks of gestation constitute risk factors for early-onset neonatal infection what indicate the need for detection and monitoring of this infections among pregnant women even before pregnancy.

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